REMARKS

Claims 1-5, 8-10, 13-15, 22 and 23 are pending in this application and stand rejected. Applicants request that the amendments in the paper filed February 2, 2004, paper no. 23, not be entered at this time. Claim 1 is amended herein and claims 8-10 are canceled without prejudice.

The Office contends that the previous amendment of paper no. 21 introduces impermissible new matter into the specification at page 14, lines 9-23. Applicants herein amend the specification to remove the matter objected to by the Office. Applicants do not admit that the matter introduced by amendment is new matter, but contend that it is the correction of an obvious mistake. The Office has pointed out evidence showing that this matter is a correction of an obvious mistake by stating that the specification indicates that "the working examples were done with media comprising 1 ng/ml GMCSF (i.e. page 16, line 17)." This statement is misleading. Although the specification does refer to experimental work performed using such media at page 16, the specification also refers to culturing cells in media containing 1 ng/ml SCF at page 20, line 25 to page 21, line 1, and the actual experimental work in working examples refers to culturing the inventive cells in SCF at 1 ng/ml (see Example 3, page 38, line 28). This shows that the first recitation of GMCSF on page 16 is an error. Nevertheless, to allow prosecution of the claims to proceed, Applicants have canceled the amendments of the previous response by amendment here.

With respect to this issue, Applicants would like to point out that the provisional application from which this application claims benefit also refers to 1 ng/ml SCF in the specification at page 46, line 20 and in the working examples at page 56, lines 10-11. Applicants therefore request that the Office acknowledge

Applicants' priority date of December 4, 1998 for at least this subject matter.

The Office expresses concern about the state of the art with respect to "the presence of SCF" to "maintain stem cells as mitotically dominant." See Office Action at page 5, lines 11-14. Although the Office did not cite any particular language from the previous response, Applicants believe that this statement refers to page 11 at lines 15-22 or page 11 at lines 31-32 of the response. Applicants did not state that it was known that the presence of SCF was necessary to maintain stem cells as mitotically dormant. Applicants stated that it was known that the presence of SCF was necessary to stem cells' survival (their state with respect to mitosis was not mentioned in the context of SCF). Applicants also stated, in a separate, numbered clause, that the art recognized that culturing stem cells in 15 ng/ml IL-3, 15 ng/ml IL-6 and 1.5 ng/ml GM-CSF would not maintain the cells as mitotically dormant.

The Office has requested that the Applicants provide prior art showing the state of knowledge with respect to this issue. In response to the Office's request, Applicants are submitting an Information Disclosure Statement, with form PTO-1449, citing three references. Applicants request that the Examiner consider these references, which were published before or near the effective filing date of this application.

Luskey et al., *Blood* 80(2):396-402, 1992, disclose that SCF in combination with IL-6 appears to "increase the survival and self-renewal" of hematopoietic stem cells. See abstract. These authors teach that the cells were dividing (page 396, col. 1, line 12) and that their chosen method of gene integration required "active cell cycle" (page 396, col. 1, line 14). Luskey et al. also teach that SCF has a proliferative effect on hematopoietic stem and progenitor cell populations (page 396,

col. 2, lines 24-25), and therefore improves retroviral-mediated gene transfer. The difficulty of achieving sufficient gene transfer in non-cycling, true hematopoietic stem cells was discussed in the present specification. See page 2-3.

The cultures of Luskey et al. contained IL-3, IL-6 and SCF in different combinations (see Table 1, page 398), but always at concentrations much higher than the present Applicants teach. See also Materials and Methods, page 397, col. 2, lines 11-16. The effects of this high concentration of SCF discussed on pages 398-399, are an increase in CFU-S_{12} (spleen colonies at day 12) (page 398, col. 1, lines 22-25), higher levels of transgene expression (page 399, col. 1, lines 3-5) and higher proviral content (page 400, col. 1, lines 22-25). But these were dependent on the "cycling status of the target cell population," (page 400, col. 2, lines 28). Thus, it is clear that Luskey et al. do not teach a method for transferring DNA into non-cycling, quiescent hematopoietic stem cells or that SCF was necessary to do so. In fact, it teaches away from using SCF because of its proliferative effects.

Verhasselt et al. (*Blood* 91(2):431-440, 1998) also cultured cells in 100 ng/ml SCF (see page 432, col. 2, lines 27-28), along with IL-3 in high concentrations. The cells were umbilical cord blood CD34++ cells which were able to differentiate into T cells NK cells and dendritic cells in organ culture, however only thymocyte progeny expressed the gene. Thus it is clear that true hematopoietic stem cells were not stably transfected since, by the authors' own admission, more than 90% of the sorted cord blood cells were not in cycle upon isolation and that the cells needed cytokine stimulation at these high levels for gene transfer, thus destroying their (90 %) quiescent status, to allow retroviral transduction. See page 433, col. 2, line 58 to page 434, col. 1, line 4.

The authors of Verhasselt et al. recognized that the cells, prior to cytokine stimulation, when they could not be transduced, were immature, but the cells which they actually transduced were not these immature, quiescent, mitotically dormant cells because the wisdom of the art at that time taught that high cytokine levels were necessary to maintain and transduce the cells. The transduced cells produced a T cell lineage but were not true hematopoietic stem cells. Moreover, Verhasselt et al. recognized that true stem cells were "less frequently or not transduced." This is due to the belief that cytokines were needed for cell survival but also destroyed their hematopoietic stem cell nature. See page 438, col. 2, line 47 to page 439, col. 1, line 53, which discusses these issues in the context of cell cycle status.

Cheng et al. were able to transduce, at low levels, cells capable of generating myeloid and β-lymphoid lineages with a retrovirus, but again, these cells are not the true hematopoietic stem cells that the present inventors have been able to transduce. The reason the cells Cheng et al. obtained were not true stem cells lay in their culturing technique, which used high concentrations of cytokines, including 100 ng/ml SCF, which at the time were believed to be necessary. See page 84, col. 2, line 63, where SCF is referred to as SLF. This represented the state of the art at the time Cheng et al. were working. SCF was recognized as an important factor to maintain the cells, but the wisdom of the art at that time taught that SCF should be used at high levels for cell maintenance and transduction, levels which the present invention discloses are too high to maintain the cells in a mitotically dormant, quiescent state. Thus it was not obvious to use a very low level as the inventors here did.

Applicants did not state and do not state that it was known prior to filing this application, that SCF was necessary to maintain quiescent, mitotically dormant hematopoietic stems. In

fact, the opposite was true. Prior to this invention, it was thought that SCF was necessary to keep the cells alive and to transduce the cells, but that it also caused differentiation, thus preventing maintenance of a mitotically dormant state and creating an apparently insurmountable barrier to successfully transducing true, quiescent stem cells. This defines the problem which the present inventors have solved.

Because Applicants have canceled the alleged new matter in the specification, Applicants now request that the Office's objection to the specification be withdrawn.

Claims 1-5, 8-10, 13-15, 17-18 and 22-23 are rejected for failing to comply with the written description requirement of 35 U.S.C. §112, first paragraph. Applicants have amended the claims to correspond to language of the specification as amended herein, to permit prosecution to progress.

The claims were rejected on grounds that adding a recitation of specific levels of SCF and deletion of reference to particular levels of GM-CSF results in a lack of support. Applicants would like to point out at the outset that claim 1 never has recited the term GM-CSF or any particular levels of GM-CSF at any stage in the prosecution of this application. Any alleged "deletion" of this term from claim 1 or any claim dependent on claim 1, directly or indirectly, therefore cannot properly be said to result in "new matter," under any circumstances.

Furthermore, the specification, as well as the original claims, supports methods of culture of hematopoietic stem cells that do not include GM-CSF or where the level of GM-CSF is zero. For example, see page 20, line 26 to page 21, line 1, which discusses cytokine concentrations for maintaining hematopoietic stem cells in which the GO, non-dividing status has been confirmed. The cytokines listed include IL-3, IL-6 and 1 ng/ml SCF, but GM-CSF is not present. As described in the

specification, the data presented in Figures 5 and 6 were obtained with cells cultured in this manner, with 1 ng/ml SCF but without GM-CSF, and show, see particularly Figure 6 and the related text, that the cells remain quiescent in the presence of these cytokines even after seven days in culture. Thus, the specification fully supports culture media which do not contain GM-CSF.

In addition, at page 29 of the specification as filed, the description clearly refers to culture in "low cytokine concentrations (10 ng/ml IL-3, 10 ng/ml IL-6 and 1 ng/ml SCF)." See lines 5-6. This recitation provides even further written description for culture of hematopoietic stem cells in media that lack GM-CSF and that contain SCF. Finally, working example 3 clearly describes transduction and culture of cells in media containing "IL-6 (10 ng/ml), IL-3 (10 ng/ml) and SCF (1 ng/ml)." Applicants submit that, even apart from showing that recitation of GM-SCF earlier in the specification on page 16 was an obvious error that should be corrected, this description provides more than adequate written support describing what is claimed here, including media that do not contain GM-CSF and do contain SCF.

With respect to the contention that the added recitation of SCF also introduced new matter, Applicants believe that the above-cited description in the specification and examples fully supports the recitation of 1 ng/ml SCF which is claimed here. Applicants therefore request that the rejection of the amended claims for lack of written description be withdrawn.

Claims 1-5, 8-10, 13-15, 17-18 and 22-23 are rejected under 35 U.S.C. §112, first paragraph, as not enabled. Specifically, the Office contends that embodiments where IL-3, IL-6 and GM-CSF are not present are not enabled. Office Action, page 8, lines 19-20. Applicants have discussed above the recitations in the body of the specification and in the working example (Example 3),

which show how to transfer DNA to primitive, in vivo-repopulating cells by transducing and then culturing the cells in IMDM containing IL-3, IL-6 and SCF. GM-CSF was not present in the working example and was not recited there. Applicants would like to point out that the Office specifically remarks that there are no working examples for practicing the methods with cultures where IL-3, IL-6 and GM-CSF are not present. This is not true with respect to GM-CSF, which is not present in working Example 3.

Therefore, Applicants believe that the specification, even if unamended to correct the error, provides full support for embodiments where GM-CSF is not present. Applicants also refer the Office to the discussions above concerning support for embodiments that do not involve GM-CSF and submit that all rejections based on lack of support for these embodiments are not properly made because the Office cannot properly restrict the claims to one set of embodiments on grounds of lack of support when others also are disclosed.

With respect to the Office's second contention, that embodiments where the cytokines "IL-3, IL-6 and GM-CSF are at higher than the cited levels," are not enabled, Applicants submit that such an embodiment is not claimed. The claims have been amended to recite cytokine levels of IL-3 and IL-6 which the Office concedes are described throughout the specification, along with levels of SCF that are recited at pages 20-21, 29 and 38.

Applicants request that the rejection of the claims as not enabled under the standards of 35 U.S.C. §112, first paragraph be withdrawn in light of the amendments and remarks herein, but reserve the right to prosecute any cancelled subject matter in a continuation and/or divisional application.

Applicants request reconsideration and allowance of the application and invite the Examiner to telephone the undersigned

should any issues remain outstanding with respect to any of the amended claims.

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